GREEN SYNTHESIS OF METAL OXIDE NANOPARTICLES: A FLEXIBLE INTERFACE FOR BIOLOGICAL, ENVIRONMENTAL AND CATALYTIC APPLICATIONS

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Introduction

"Nanotechnology is the application of science to control matter at the molecular level" [1]. Nanotechnology serves at various fields of science and technology at different aspects of fundamental and applied frontiers in engineering and material science. Nanotechnology currently being a major research area has an impressive influence on human health care aspects. For the comparison purpose, the size of the nano regime particles is 100 to 10,000-fold smaller than the bio molecules like enzymes, nucleic acids, receptors etc. The small size, large surface area, morphology, optical, mechanical, electrical properties make these nanoparticles employable in various fields of medicine, chemistry, opto-electronics, energy etc. [2].

Introduction on Metal Oxide nanoparticles: Metal oxide nanoparticles (MO NPs) play a significant role in various fields of physics, chemistry, and material science. They manifest vast structural geometries with corresponding electronic structure. These MO NPs can be fabricated to find employability in several devices including fuel cells, piezoelectric, coatings, sensors, microelectronic circuits etc. The utility of nanotechnology field is in fabricating nanostructures incorporated with unique properties than that of its single particle or bulk matter. The nano regime size of these unique structures exhibits large density of surface-active sites, hence no wonder they possess extra ordinary physical and chemical properties. The size of the particle definitely influences its structural characterization and electronic properties. The nano-regime size also effects the lattice symmetry in the cell parameter etc. This largely make them different than that of the bulk matter, where the structural properties are quite stable. The increase in the strain with increased surface energy enabling the "dangling bond" formation, with decreased particle size is of great significance for finding unique applications of these MO NPs. The "Quantum confinement" effect of MO NPs arising in the electronic states (like that of discreate atom -like electronic states) largely influence its optical properties, particularly in photocatalytic applications [3]. The unique features of these MO NPs such as magnetic, optical, and structural majorly depends on its large surface to volume ratio. The oxide of metals like copper, iron, zinc, cobalt finds applicability in semiconductor devices, electronics, solar cells, catalysis etc. [4].

Green synthesis of MO NPs: "Green Chemistry" is a transpiring branch of Chemistry, which endeavors on Biogenic, cost-effective, eco-friendly production of most stable MO NPs which are profoundly studied due to its usual exclusive optical, electrical, chemical, and catalytic

properties. Green synthetic strategy of MO NPs synthesis via diverse Micro-organisms (Bacteria, Actinomycetes, Fungi, Yeast, Algae), medicinally enriched plant materials such as *Oscimum sanctum, Terminalia chebula, Hordeum Vulgare, Rumex acetosa, Solanum tricobatum, Syzygium cumini, Centella asiatica, Citrus sinensis, Curcuma longa* and so on are reported attributed with additional biological functions. The secondary metabolites in the biological agents act as environmentally amiable Natural reductant comprising Alkaloids, Flavonoids, Saponins, Steroids, Tannins, and other nutritional compounds, which allow complete elimination of non-toxic agents for stabilization of Nanoparticles are responsible for the complete reduction of the Metal precursor to well-characterized MO NPs. The complete mechanism of bio-reduction of Metal ions by plant materials is not yet clearly understood and hence gaining much attention of researchers. Currently, the use of natural organic extracts such as plant and microorganism extracts has gained its importance not only in the synthesis of pure single metallic oxides nanoparticles, but have been used also in the preparation of doped, bimetallic oxide NPs [5].

Photocatalytic applications

Nanomaterials have been attracting the attention of the researcher's worldwide due to the employability of these nano regime materials in the technologies involved in the water purification process. A review report by Wadhawan, S et al. (2020) presents the role of well-characterized nano sorbents with tunable surface area, adsorption capacity for heavy metal pollutants from water source [6]. Inorganic nanomaterials such as carbon-based nanomaterials, oxides of transition metal-based nanomaterials, nanomembranes, organic polymer-based nanomaterials are widely used nanotechnology-based systems for the purpose [7]. The harmful chemicals and non-green solvents with the role as reducing agent, the surface modifying and functionalization of the materials intended to process these nanomaterials again put the risk of water contamination.

Several literatures are available where Phyto reduced and functionalized NPs are used in catalysis and degradation of several textile dye pollutants in the aqueous water system and thus their applicability in the environment remediation. Dangling bonds present at the surface of the MO NPs which are unsaturated are available for functionalization of the NPs during the initial reduction process [8]. Thus, it may be believed that the phytochemicals present in the plant crude extract are not only used in the reduction process alone but also have a definite role in coordination with the unsaturated bonds at the surface of the nanoparticles and hence its

functionalization. These functionalized nanoparticles are used as photocatalysts. These phytochemical functionalized nanostructures will degrade dye pollutants and imparting it into harmless by-products.

Biomedical applications

In pharmaceutical industries, innovative research in nanoscience has been carried out for the diagnosis and therapy of various kinds of diseases. According to a review by Kumar et al. 2013, reports various nano-fabrications in the medicinal field with enhanced therapeutic value compared to conventional medicines. These nanomedicines find their applications balancing their efficacy and toxicity and contribute to the human health care prospects [9]. The MO NPs like iron oxide, gold, etc., are advantageous in developing drug delivery systems that can be controlled by external signals. The plethora of other applications of various MO NPs include antibacterial, anticancer, anti-biofilm, anthelmintic, biocatalytic, larvicidal activities etc. [10]. Although there are excellent opportunities for metal-based NPs in the medical field as therapeutics and in disease diagnosis, its regulation is important, and various initiatives are taken by the Environmental Protection Agency (EPA), Health and Consumer Protection Directorate of the European Commission and US Food and Drug Administration (FDA) for the safe administration and to eliminate the risk of health hazards posed by NPs [11]. Phyto-nano therapy finds a solution to the applicability of MO NPs by reducing the toxic effects and enhancing biocompatibility. Several bio-synthesized nanoparticles like ZnO, α-Fe_2O_3, Au, Ag, CuO etc., find applications in biomedical areas with bio-compatibility inheritance.

Electrochemical applications

The information regarding the electrochemical reactions that occur in the fabricated electrode can be gathered from the electrochemical study. The cyclic voltammetry and Electrochemical Impedance spectra serve as two major powerful tools, that provide information on the redox reactions, its kinetics, capacitance, charge transfer resistance etc. Multifunctional MO NPs is fabricated as electroactive material in sensing numerous compounds, including nitrobenzene, dopamine etc. We have chosen the Paracetamol drug to evaluate the fabricated NPs sensing ability in the present study. Paracetamol is widely used as an antipyretic analgesic drug, which is used in the control of body temperature. The overdose of the drug may result in side effects as it leads to the accumulation of toxic metabolites [12]. Thus, the accurate, rapid detection of

the Paracetamol drug is essential, and the electrochemical approach is gaining much interest in recent days.

The present thesis work includes synthesis of green synthesized MO NPs (CuO, α-Fe_2O_3 and CeO_2) from two plant leaves extract i) *Scoparia dulsis L*. and ii) *Pouteria campechiana L.*. The both plant is chosen as it is of great medicinal value.

Scoparia dulsis L. (SD): SD is a flowering herbal plant belonging to family *Plantaginaceae*, distributed almost around the globe. The plant is of medicinal importance and is traditionally used for many human disorders including diabetes mellitus, hypertension, ulcers, cancer, wounds, skin rash, cough, and tuberculosis, etc. The plant is rich in carbohydrates, saponins, flavonoids, alkaloids, steroids tannins, and terpenes as revealed from the phytochemical analysis of the same.

Pouteria campechiana (PC): PC leaf extract is used as a reducing and functionalizing agent. PC is tropical and subtropical fruit tree commonly called Canistel, commonly found native to Central and South America belonging to family Sapotaceae. The plant constitutes poly phenolic phytoconstituents such as gallic acid, (+)-gallocatechin, (+)-catechin, (−)-epicatechin, dihydromyricetin, and (+)-catechin-3-O-gallate. The leaves of Canistel species are believed to possess anti-inflammatory activity. Along with these benefits it offers antipyretic, in ulcer treatment and in control of diarrhoea.

The prepared NPs were characterized by analytical and microscopic tools like, FT-IR, p-XRD, UV Vis/DRS, FESEM-EDS and BET-BJH analysis. The prepared biogenic NPs find potent applications in the field of photocatalysis, biomedical and electrochemistry for paracetamol sensing. This thesis work includes eight chapter, including six working chapters.

Chapter 1: General Introduction

Chapter 2: Experimental Section

- Characterization Techniques
- Phytochemical analysis of Plant material

Chapter 3: Phyto assisted synthesis and characterization of Scoparia dulsis L. leaf extract mediated porous nano CuO for photocatalytic and biomedical applications

- Phyto synthesis of Nanoparticles
- Characterization
- Photocatalytic applications
- Bio medical applications

Chapter 4: Phyto assisted synthesis and characterization of Pouteria campechiana L. leaf extract mediated porous nano CuO for photocatalytic and biomedical applications

- Phyto synthesis of Nanoparticles
- Characterization
- Photocatalytic applications
- Bio medical applications

Chapter 5: Bio-fabrication of multifunctional quasi-spherical green α-Fe_2O_3 nanostructures for biomedical applications and Paracetamol sensing by electrochemical studies.

- Phyto synthesis of Nanoparticles
- Characterization
- Bio medical applications
- Electrochemical applications

Chapter 6: Synthesis, characterization of Phyto-functionalized nano hematite from extract of *Pouteria campechiana* L. for lung cancer suppressive activity and Paracetamol sensing by electrochemical studies

- Phyto synthesis of Nanoparticles
- Characterization
- Bio medical applications
- Electrochemical applications

Chapter 7: Phyto assisted synthesis and characterization of *Scoparia dulsis L*. leaf extract mediated porous nano CeO_2 for electrochemical and biomedical applications

- Phyto synthesis of Nanoparticles
- Characterization
- Bio medical applications
- Electrochemical applications

Chapter 8: Phyto synthesis, characterization of multifarious nano-ceria from *Pouteria campechiana L*. for biomedical and Paracetamol sensing applications

- Phyto synthesis of Nanoparticles
- Characterization
- Bio medical applications
- Electrochemical applications

Thesis Organization

Chapter 1:

Chapter 1 deals with general introduction on green synthesis of NPs, its properties and gives brief literature survey on various Phyto synthesized NPs and their applications.

Chapter 2:

Chapter 2 describes the experimental section, brief introduction on various characterization techniques. The phytochemical analysis of the plant material (*Scoparia dulsis* L. and *Pouteria campechiana*) chosen has also explained in this section.

Chapter 3:

Phyto assisted synthesis and characterization of *Scoparia dulsis L.* leaf extract mediated porous nano CuO for photocatalytic and biomedical applications

In this chapter, we present the synthesis of series of copper oxide nanostructures with porous nature using very rapid and efficient Solution Combustion Synthesis (SCS) methodology. The synthesis utilizes various volumes of crude leaves extract of *Scoparia dulsis L.* (SD). that has definite role in directing the particle size of copper oxide nanoparticles (CSD NPs) during the reduction process. The CSD NPs are well characterized by the various analytical and microscopic tools including FT-IR, p-XRD, FESEM-EDS, UV-Vis and BET-BJH analysis. The porous morphology of the nanostructures and the elemental composition purity are evident from the micrographs obtained from Field Emission Scanning Electron Microscopy (FESEM) coupled with Energy Dispersive Spectroscopy (EDS). UV–Vis spectroscopic analysis was carried out and band gap via Tauc-plot relation and was reported. A large surface to volume ratio and the pore volume of CSD NPs was observed by Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) analysis. The prepared nanoparticles show enhanced photocatalytic study rendering two model dyes (Methylene blue and Methyl orange) to colorless degraded products within 180 min with reusability over 5 cycles owing to its stability. The *in vitro* antioxidant behavior of CSD NPs is reported. Significant *in vitro* anti-inflammatory behavior is observed for all CSD NPs with % protective action against Human Red Blood cells. The CSD NPs proved significant cytotoxicity effect against adenocarcinomic human alveolar basal epithelial cells. Thus, the CSD NPs are believed to possess multifaceted

applications in environmental and biomedical fields. The results obtained has been reported and discussed in detail.

Chapter 4:

Phyto assisted synthesis and characterization of *Pouteria campechiana L.* leaf extract mediated porous nano CuO for photocatalytic and biomedical applications

In this scientific paper we report, cost-effective synthesis of unique copper oxide nanoparticles (CPC NPs) coupled with properties from phytocompounds of *Pouteria campechiana L.* and copper metal precursor with variegated morphology. The synthesis included variation in the volume of extract, keeping amount of metal precursor constant and adapting Solution Combustion Synthesis. Various microscopic and analytical techniques were adapted for the characterization of rice grain shaped CPC NPs. The CSD NPs are well characterized by the various analytical and microscopic tools including FT-IR, p-XRD, FESEM-EDS, UV-DRS and BET-BJH analysis. The prepared nanoparticles showed enhanced photocatalytic property rendering two model dyes (Methylene blue and Methyl orange) to colorless degraded products within 180 min with reusability over 5 cycles owing to its stability. The invitro antioxidant behaviour of CSD NPs is reported. The *in vitro* anti-inflammatory results of CPC NPs proved to be exhibit prominent protective action for human RBC owing to its biocompatibility. *In vitro* anticancer activity studies of CPC NPs against adenocarcinoma human alveolar basal epithelial cells have proven to show the cytotoxic effect with appreciable IC50 value. Thus, the CPC NPs are believed to possess multifaceted applications in environmental and biomedical fields. The results obtained has been reported and discussed in detail.

Chapter 5:

Bio-fabrication of multifunctional quasi-spherical green α-Fe_2O_3 nanostructures for biomedical applications and Paracetamol sensing by electrochemical studies.

Green metal oxide nanostructures with various diverse properties are synthesized to find solutions to various problems arising in the field of medicine and technology. The present study includes cost-effective, eco-friendly synthesis of α-Fe_2O_3 nanoparticles (ISD NPs), using varied volumes of extract of *Scoparia dulsis L*. as reducing agent via Solution combustion synthesis. The ISD NPs are well characterized by the various analytical and microscopic tools including FT-IR, p-XRD, FESEM-EDS, UV-Vis and BET-BJH analysis. The extract

influences the size, shape of quasi-spherical, bandgap positions, surface area to volume ratio of the nanoparticles prepared. The structural purity is ascertained via p-XRD studies. The optical energy gap and the mesoporous nature of the biogenic NPs are attributed UV-DRS and surface area studies. The characterization findings reveal the "symbiotic association" of bio reducing agent along with metal to influence the pronounced activity in biomedical and electrochemical studies. The controlled size and morphology give explicit results in in-vitro assays performed. The free radical scavenging hunt was maximum with DPPH inhibition which attribute to the in-vitro antioxidant study. The inherent stabilization behavior of ISD NPs towards human RBC's is appreciated, owing to its bio-compatibility nature. The ISD NPs exhibited depreciated cell viability of adenocarcinoma human alveolar basal epithelial cells with a lower IC50 value. The moderate antidiabetic property via pancreatic α-amylase inhibition was observed for ISD NPs. The electrochemical studies of ISD NPs were attributed by: i) Cyclic voltammetry studies ii) Electrochemical Impedance study. The ISD NPs as bio electrodes is a promising sensor for paracetamol drug detection as indicated by the electrochemical studies. Thus, the ISD NPs are believed to possess multifaceted applications in biomedical and electrochemical fields. The results obtained has been reported and discussed in detail.

Chapter 6:

Synthesis, characterization of Phyto-functionalized nano hematite from extract of *Pouteria campechiana L.* for lung cancer suppressive activity and Paracetamol sensing by electrochemical studies

This chapter describes the synthesis of hematite NPs (IPC NPs) from *Pouteria campechiana L.*extract-based reducing agent. The influence of extract on the size and morphology of the NPs was known by varying the volume of the extract during the synthesis by Solution Combustion Synthesis. The IPC NPs were characterized by FT-IR, p-XRD, FESEM-EDS, UV-DRS, and BET-BJH analysis. The extract influences the size, shape of quasi-spherical, bandgap positions, surface area to volume ratio of the nanoparticles prepared. The DPPH scavenging activity was recorded and IC50 value was calculated. The invitro Human Red Blood Cells Stabilization assay carried out has evinced the haemocompatibility of hematite NPs. Furthermore, the in-vitro cytotoxicity study has manifested the A549 lung cancer suppressive activity of IPC NPs with a decreased cell viability and low IC50 values. Additionally, the NPs also showed a dose-dependent invitro α- amylase inhibition activity. The

synthesized biogenic IPC NPs exhibited enhanced electrochemical studies of IPC NPs evaluated by: i) Cyclic voltammetry studies ii) Electrochemical Impedance study. The IPC NPs as bio electrodes is a promising sensor for paracetamol drug detection as indicated by the electrochemical studies. Hence these IPC NPs are promising materials in the field of pharmaceuticals and biomedical fields. The results obtained has been reported and discussed in detail.

Chapter 7:

Phyto assisted synthesis and characterization of *Scoparia dulsis L.* leaf extract mediated porous nano CeO_2 for electrochemical and biomedical applications

This chapter focuses on the synthesis of size-controlled CeO_2 (CeSD NPs) from aqueous extract of *Pouteria campechiana L.* via self-propagating solution combustion synthesis. The extract volume is varied to know the influence of extract on the physical, optical and surface properties of CeSD NPs. The bio-fabricated NPs are well characterized by FT-IR, p-XRD, FESEM-EDS, UV-DRS and BET-BJH techniques. The p-XRD study revealed the size-controlled synthesis of CeSD NPs from the plant extract chosen. The large specific surface area of with a maximum pore diameter of nm was observed for CeSD NPs. The antioxidant behavior was maximum via DPPH inhibition at high concentration. The bio-derived NPs shows appreciative invitro anti-inflammatory via human red blood corpuscles membrane protection owing to its non-toxic behavior. In vitro A546 cancer suppressive activity of CeSD NPs reveals that phytochemicals functionalized by highest volume during synthesis showed a low IC50 value despite the large size. The pancreatic α-amylase inhibition activity of CeSD was found to increase in dose-dependent manner. The CeSD NPs exhibits suitable antibacterial applications against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas syringae* and *Pseudomonas aeruginosa*. The synthesized biogenic CeSD NPs exhibited enhanced electrochemical studies of CeSD NPs evaluated by: i) Cyclic voltammetry studies ii) Electrochemical Impedance study. The CeSD NPs as bio electrodes is a promising sensor for paracetamol drug detection as indicated by the electrochemical studies. And hence the bio electrodes have superlative efficiency in sensing the Paracetamol drug. The results obtained has been reported and discussed in detail.

Chapter 8:

Phyto synthesis, characterization of multifarious nano-ceria from *Pouteria campechiana L.* for biomedical and Paracetamol sensing applications

This chapter focuses on the synthesis of size-controlled nano-ceria (CePC NPs) from aqueous extract of *Pouteria campechiana* via self-propagating solution combustion synthesis. The extract volume is varied to know the influence of extract on the physical, optical and surface properties of CePC NPs. The bio-fabricated NPs are well characterized by FT-IR, p-XRD, FESEM-EDS, UV-DRS and BET-BJH techniques. The p-XRD study revealed the size-controlled synthesis of CePC NPs from the plant extract chosen. The large specific surface area of with a maximum pore diameter of nm was observed for CePC NPs. The antioxidant behavior was maximum via DPPH inhibition at high concentration. The bio-derived NPs shows appreciative invitro anti-inflammatory via human red blood corpuscles membrane protection owing to its non-toxic behavior. In vitro A546 cancer suppressive activity of CePC NPs reveals that phytochemicals functionalized by highest volume during synthesis showed a low IC50 value despite the large size. The pancreatic α-amylase inhibition activity of CePC was found to increase in dose-dependent manner. The CePC NPs exhibits suitable antibacterial applications against Bacillus subtilis, Staphylococcus aureus, Pseudomonas syringae and Pseudomonas aeruginosa. The synthesized biogenic CePC NPs exhibited enhanced electrochemical studies of CePC NPs evaluated by: i) Cyclic voltammetry studies ii) Electrochemical Impedance study. The CePC NPs as bio electrodes is a promising sensor for paracetamol drug detection as indicated by the electrochemical studies. And hence the bio electrodes have superlative efficiency in sensing the Paracetamol drug. The results obtained has been reported and discussed in detail.

Summary and Conclusions:

The synthesized biosynthesized NPs were well characterized via different analytical and microscopic techniques. The NPs were evaluated for various applications including photocatalytic, biomedical, and electrochemical studies. The conclusions drawn from whole study has been reported.

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List of Publications

1. K.M. Navada, G.K. Nagaraja, J.N. D'Souza, S. Kouser, R. Ranjitha, D.J. Manasa, Phyto assisted synthesis and characterization of Scoparia dulsis L. leaf extract mediated porous nano CuO photocatalysts and its anticancer behavior, Appl. Nanosci. 10 (2020) 4221–4240. https://doi.org/10.1007/s13204-020-01536-2.
2. K.M. Navada, N.G.K. Ranjitha, R.J. Neetha, D.S. Sabia, K. Manasa, Synthesis, characterization of phyto - functionalized CuO nano photocatalysts for mitigation of textile dyes in waste water purification , antioxidant , anti - inflammatory and anticancer evaluation, Appl. Nanosci. 11 (2021) 1313–1338, https://doi.org/10.1007/s13204-021-01688-9. https://doi.org/10.1007/s13204-021-01688-9.
3. K.M. Navada, G.K. Nagaraja, J.N. D'Souza, S. Kouser, C.R. Ravikumar, D.J. Manasa, Bio-fabrication of multifunctional quasi-spherical green α-Fe2O3 nanostructures for paracetamol sensing and biomedical applications, Ceram Int. 47 (2021) 33651–33666. https://doi.org/https://doi.org/10.1016/j.ceramint.2021.08.275.

Papers under communication

1. K.M. Navada, G.K. Nagaraja, J.N. D'Souza, S. Kouser, C.R. Ravikumar, D.J. Manasa, Synthesis, characterization of Phyto-functionalized nano hematite for lung cancer suppressive activity and Paracetamol sensing by electrochemical studies – Under Review
2. K.M. Navada, G.K. Nagaraja, J.N. D'Souza, S. Kouser, B.R. Nithyashree, D.J. Manasa, Phyto synthesis, characterization of multifarious nano-ceria for biomedical and sensing applications – Accepted (Journal of Photochemistry and Photobiology A: Chemistry)
3. K.M. Navada, G.K. Nagaraja, J.N. D'Souza, S. Kouser, Jyothi K, D.J. Manasa, Phyto assisted synthesis and characterization of nano CeO_2 mediated from *Scoparia dulsis L*. leaf for biomedical and sensing applications-Communicated

Papers presented in conferences

1. Meghana Navada K and G.K Nagaraja (2019) "Environmetally benign, green synthesis of monoclinic Copper oxide nanoparticles and its Characterization" International conference on Advanced Functional Materials for Energy, Environment and Health care, held at Mysore during 18-20th March 2019 (Poster)
2. Meghana Navada K and G.K Nagaraja "Size controlled green synthesis of SD extract capped monoclinic Copper oxide Nanoparticles: A promising photocatalysts in dye

degradation studies" three-day International conference on Advances in Chemical and Material Sciences held at Mangalore University, Mangalore during October, 17-19th 2019. (Poster)

3. Meghana Navada K , G.K.Nagaraja, Josline Neetha D'Souza, Sabia Kouser, "Synthesis of Nano CuO: Optical, Structural, Surface characterizations "one day National conference on Novel Materials and Devices for Future Applications (NMDFA-2020), held at held at St. Aloysius College (Autonomous), Mangalore on February, 18th 2020. (Poster)
4. Meghana Navada K and G.K Nagaraja "Controlled synthesis of nano α-Fe2O3 mediated from *Scoparia dulsis L.*: Optical, Structural, Surface characterizations" two-dayInternational Virtual Conference on Emerging Trends in Nanoscience and Nanotechnology held at Srinivas University, Mangalore during August, 5-6th 2021 (Oral)

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